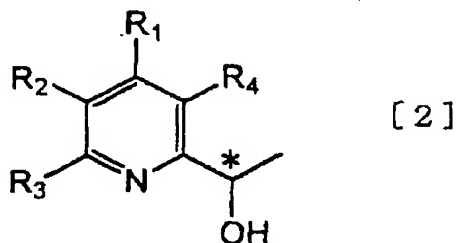


## CLAIMS

1. A method of producing an optically active pyridineethanol derivative represented by the general formula

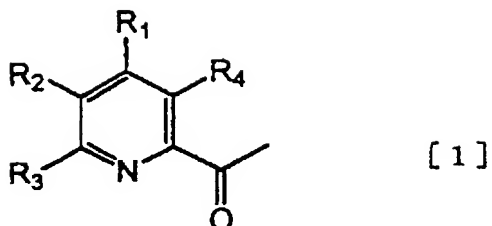
5 [2]:



wherein  $R_1$  and  $R_2$  are bound to each other to form a 5- to 8-membered monocyclic heterocycle containing at least one hetero atom  
 10 selected from the group consisting of oxygen, sulfur and nitrogen atoms, which heterocycle may optionally have a substituent(s), or a polycyclic heterocycle resulting from the condensation of such monocyclic heterocycle with another ring, which polycyclic heterocycle may optionally have a  
 15 substituent(s),

$R_3$  and  $R_4$  are the same or different and each represents a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon  
 20 atoms, which may optionally have a substituent(s), and \* indicates that the asterisked carbon atom is an asymmetric one,

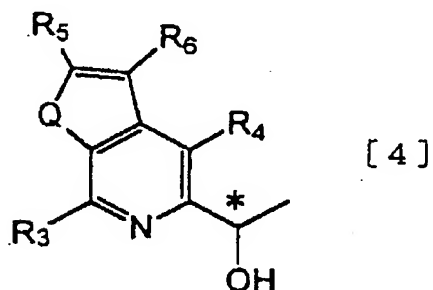
which method comprises stereoselectively reducing an acetylpyridine derivative represented by the general formula  
 25 [1]:



wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above,  
by causing an enzyme or enzyme source capable of asymmetrically  
reducing the same to act thereon.

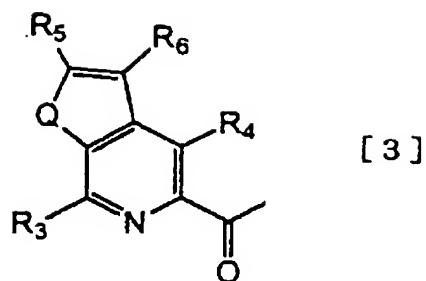
5

2. A method of producing an optically active  
pyridineethanol derivative represented by the general formula  
[4]:



10 wherein Q represents an oxygen or sulfur atom or a group of the  
general formula  $-N(D)-$ , in which N is a nitrogen atom and D  
represents a hydrogen atom or a monovalent protective group,  
 $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are the same or different and each represents  
a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group  
15 containing 1 to 12 carbon atoms, which may optionally have a  
substituent(s), or an alkoxy group containing 1 to 12 carbon  
atoms, which may optionally have a substituent(s),  
and \* indicates that the asterisked carbon atom is an asymmetric  
one,

20 which method comprises stereoselectively reducing an  
acetylpyridine derivative represented by the general formula  
[3]:



wherein Q, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are as defined above,  
by causing an enzyme or enzyme source capable of asymmetrically  
reducing the same to act thereon.

5

3. The production method according to Claim 2,  
wherein Q is an oxygen atom.

10

4. The production method according to Claim 2,  
wherein Q is an oxygen atom,  
R<sub>3</sub> is a hydrogen atom or a chlorine atom,  
R<sub>4</sub> is a hydrogen atom,  
R<sub>5</sub> is a hydrogen atom  
and R<sub>6</sub> is a hydrogen atom or a methyl group.

15

5. The production method according to Claim 2,  
wherein Q is an oxygen atom  
and R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> each is a hydrogen atom.

20

6. The production method according to any of Claims 1  
to 5,

25

wherein the reaction is carried out in the presence of  
an enzyme capable of reducing the oxidized form nicotinamide  
adenine dinucleotide and/or the oxidized form nicotinamide  
adenine dinucleotide phosphate to the respective reduced forms  
as well as a substrate for the reduction.

7. The production method according to Claim 6,  
wherein said enzyme for reduction to the reduced form is

glucose dehydrogenase

and said substrate for reduction is glucose.

8. The production method according to Claim 6,  
5 wherein said enzyme for reduction to the reduced form is  
formate dehydrogenase  
and said substrate for reduction is formic acid.

9. The production method according to any of Claims 1  
10 to 8,

wherein said enzyme or enzyme source is derived from a  
microorganism selected from the group consisting of  
microorganisms of the genera Ashbya, Candida, Cryptococcus,  
Clavispora, Debaryomyces, Dipodascus, Galactomyces,  
15 Geotrichum, Guilliermondella, Hanseniaspora, Hansenula,  
Hyphopichia, Issatchenkia, Kluyveromyces, Kuraishia,  
Lodderomyces, Metschnikowia, Ogataea, Pachysolen, Pichia,  
Rhodospiridium, Rhodotorula, Saccharomycopsis,  
Schwanniomyces, Sporidiobolus, Sporobolomyces,  
20 Schizoblastosporion, Stephanoascus, Torulaspora, Trigonopsis,  
Trichosporon, Willopsis, Yamadazyma, Zygosaccharomyces,  
Alcaligenes, Bacillus, Brevibacterium, Cellulomonas,  
Corynebacterium, Jensenia, Ochrobactrum, Pseudomonas,  
Rhodococcus and Tsukamurella.

25 10. The production method according to Claim 9,  
wherein the product optically active pyridineethanol  
derivative has the S absolute configuration

and said enzyme or enzyme source is derived from a  
30 microorganism selected from the group consisting of  
microorganisms of the genera Ashbya, Candida, Cryptococcus,  
Clavispora, Debaryomyces, Dipodascus, Galactomyces,  
Geotrichum, Guilliermondella, Hanseniaspora, Hansenula,  
Hyphopichia, Issatchenkia, Kluyveromyces, Kuraishia,  
35 Lodderomyces, Metschnikowia, Ogataea, Pachysolen, Pichia,

Rhodosporidium, Rhodotorula, Saccharomycopsis,  
Schwanniomyces, Sporidiobolus, Sporobolomyces,  
Schizoblastosporion, Stephanoascus, Torulaspora, Trigonopsis,  
Trichosporon, Willopsis, Yamadazyma, Zygosaccharomyces,  
5 Alcaligenes, Bacillus, Brevibacterium, Cellulomonas,  
Corynebacterium, Jensenia, Ochrobactrum, Pseudomonas,  
Rhodococcus and Tsukamurella.

11. The production method according to Claim 9,  
10 wherein the product optically active pyridineethanol  
derivative has the R absolute configuration  
and said enzyme or enzyme source is derived from a  
microorganism selected from the group consisting of  
microorganisms of the genera Candida, Ogataea, Pichia,  
15 Yamadazyma, Brevibacterium and Corynebacterium.

12. An enzyme having the following physical and chemical  
properties (1) to (3):  
(1) Activity: It stereoselectively reduces 5-  
20 acetylfuro[2,3-c]pyridine, in the presence of reduced form  
nicotinamide adenine dinucleotide as a coenzyme, to give 5-  
(1-(R)-hydroxyethyl)furo[2,3-c]pyridine;  
(2) Specificity: It has reducing ability against ketones and  
aldehydes but is very low in reducing activity against  
25 carbocyclic ketones and the  $\alpha$ -position keto group of  $\alpha$ -keto  
acids;  
(3) Molecular weight: It shows a molecular weight of about  
60,000 in gel filtration analysis and a molecular weight of  
about 29,000 in SDS polyacrylamide electrophoresis.

30

13. The enzyme according to Claim 12  
which has the following physical and chemical properties  
(4) to (6):  
(4) Optimal temperature: 50 °C to 55 °C;  
35 (5) Optimal pH: 5.0 to 6.0;

(6) Inhibitor: It is inhibited by the mercury ion.

14. An enzyme specified below under (a) or (b):

(a) An enzyme comprising an amino acid sequence shown under  
5 SEQ ID NO:1 in the sequence listing;

(b) An enzyme comprising an amino acid sequence derived from  
the amino acid sequence shown under SEQ ID NO:1 in the sequence  
listing by deletion, substitution and/or addition of one or  
several amino acids and having an activity by which 5-  
10 acetylfuro[2,3-c]pyridine is stereoselectively reduced to  
5-(1-(R)-hydroxyethyl)furo[2,3-c]pyridine.

15 15. The enzyme according to any of Claims 12 to 14  
which is derived from a microorganism belonging to the  
genus Candida.

16. The enzyme according to any of Claims 12 to 14  
which is derived from Candida maris.

20 17. The enzyme according to any of Claims 12 to 14  
which is derived from Candida maris IFO 10003.

18. The production method according to any of Claims 1  
to 8,  
25 wherein said enzyme is defined according to any of Claims  
12 to 17  
and the product optically active pyridineethanol  
derivative has the R absolute configuration.

30 19. A DNA coding for the enzyme according to any of Claims  
14 to 17.

20. A DNA comprising a base sequence shown under SEQ ID  
NO:2 in the sequence listing.

21. A recombinant vector containing the DNA according to Claim 19 or 20.

5           22. The recombinant vector according to Claim 21 which is pNTFP.

23. The recombinant vector according to Claim 21 which comprises a DNA coding for glucose dehydrogenase.

10           24. The recombinant vector according to Claim 23, wherein said glucose dehydrogenase is derived from Bacillus megaterium.

15           25. The recombinant vector according to Claim 24 which is pNTFPG.

26. A transformant having the recombinant vector according to any of Claims 21 to 25.

20           27. The transformant according to Claim 26, wherein the host is Escherichia coli.

28. The transformant according to Claim 27 which is Escherichia coli HB101 (pNTFP).

25           29. The transformant according to Claim 27 which is Escherichia coli HB101 (pNTFPG).

30           30. A transformant having a first recombinant vector containing the DNA according to Claim 19 or 20 and a second recombinant vector containing a DNA coding for glucose dehydrogenase.

35           31. The transformant according to Claim 30, wherein said first recombinant vector is pNTFP

and said glucose dehydrogenase is derived from Bacillus megaterium.

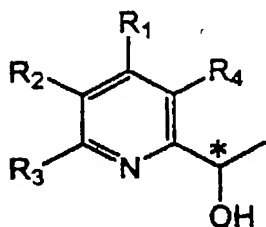
32. The transformant according to Claim 30 or 31,  
5 wherein the host is Escherichia coli.

33. The production method according to any of Claims 1  
to 5,

wherein said enzyme is the transformant according to any  
10 of Claims 26 to 32

and said product optically active pyridineethanol  
derivative has the R absolute configuration.

34. A method of producing an optically active  
15 pyridineethanol derivative having the S absolute configuration  
and represented by the general formula [6]:



[6]

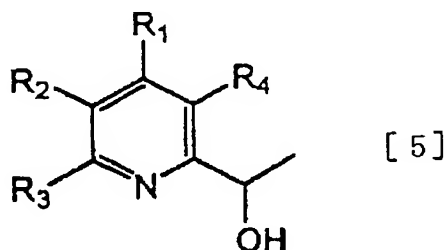
wherein R<sub>1</sub> and R<sub>2</sub> are bound to each other to form a 5- to 8-membered  
20 monocyclic heterocycle containing at least one hetero atom  
selected from the group consisting of oxygen, sulfur and  
nitrogen atoms, which heterocycle may optionally have a  
substituent(s), or a polycyclic heterocycle resulting from the  
condensation of such monocyclic heterocycle with another ring,  
25 which polycyclic heterocycle may optionally have a  
substituent(s),

and R<sub>3</sub> and R<sub>4</sub> are the same or different and each represents a  
hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group  
containing 1 to 12 carbon atoms, which may optionally have a  
30 substituent(s), or an alkoxy group containing 1 to 12 carbon



atoms, which may optionally have a substituent(s),  
and \* indicates that the asterisked carbon atom is an asymmetric  
one,

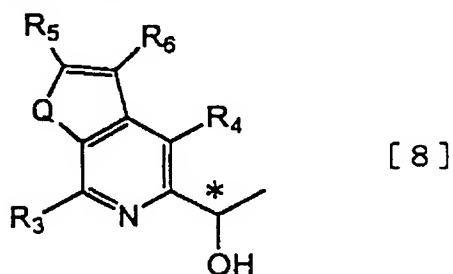
- 5 which method comprises causing the enzyme according to  
any of Claims 12 to 17 and/or the transformant according to any  
of Claims 26 to 32 to act on a pyridineethanol derivative  
represented by the general formula [5]:



- 10 wherein  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  are as defined above,  
to thereby preferentially oxidize the R form of the  
pyridineethanol derivative  
and recovering the remaining S form of the  
pyridineethanol derivative.

15

35. A method of producing an optically active  
pyridineethanol derivative having the S absolute configuration  
and represented by the general formula [8]:

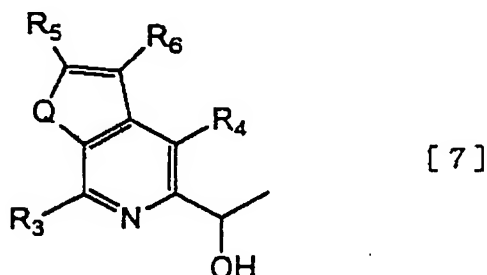


- 20 wherein Q represents an oxygen or sulfur atom or a group of the  
general formula -N(D)-, in which N is a nitrogen atom and D  
represents a hydrogen atom or a monovalent protective group,  
 $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are the same or different and each represents

a hydrogen atom, a halogen atom, a hydroxyl group, an alkyl group containing 1 to 12 carbon atoms, which may optionally have a substituent(s), or an alkoxy group containing 1 to 12 carbon atoms, which may optionally have a substituent(s),

5 and \* indicates that the asterisked carbon atom is an asymmetric one,

which method comprises causing the enzyme according to any of Claims 12 to 17 and/or the transformant according to any of Claims 26 to 32 to act on a pyridineethanol derivative  
10 represented by the general formula [7]:



wherein Q, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are as defined above,

15 to thereby preferentially oxidize the R form of the pyridineethanol derivative

and recovering the remaining S form of the pyridineethanol derivative.

20 36. The production method according to Claim 35, wherein Q is an oxygen atom.

37. The production method according to Claim 35, wherein Q is an oxygen atom,

R<sub>3</sub> is a hydrogen atom or a chlorine atom,

25 R<sub>4</sub> is a hydrogen atom,

R<sub>5</sub> is a hydrogen atom

and R<sub>6</sub> is a hydrogen atom.